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THE STANDARDISATION OF TUBERCULIN AND THE PRECIPITIN TEST.

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THE testing of tuberculin by methods based on the official test in use at Frankfort has been carried on in England for many years. Eagleton and Baxter (1923) used the official Frankfort method, in which varying doses of tuberculin are injected subcutaneously into different tuberculous guinea-pigs until the lethal dose is found; they added the von Pirquet and intracutaneous tests and thereby considerably improved the method.

With the methods at present available the degree of accuracy attainable is of a different order from that ordinarily obtained in the testing of diphtheria and tetanus toxins and their antitoxic sera, which lend themselves to accurate titration, but the test nevertheless distinguishes at least a "satisfactory" from a "useless" tuberculin. It is probable that an experienced worker can differentiate, on a short range of tests, between three tuberculins of values 100 per cent.—i.e., equal to standard tuberculin—60 and 30 per cent.; on a long range of tests smaller differences probably can be detected.

The standard tuberculins used in the work in England have been based upon those of Frankfort since the receipt of the first standard from Ehrlich several years before the war, and many different batches of tuberculous guinea-pigs have been used with fairly consistent results.

Dreyer and Vollum (1924) described a precipitin test and found that considerable discrepancies might exist between the results obtained by the guinea-pig method and the precipitin test. Table I. summarises these results.

TABLE I.

A = Description in Table VII. (Dreyer and Vollum, 1924).
B = Value by precipitin test (Dreyer and Vollum).
C = Value by guinea-pig test (Okell and Parish).

A.	B.	C.	A.	B.	C.
British 3	33·2	100	British 2	135·1	100
„ 4	46·0	100	„ 8	119·6	100
„ 5	244·7	100	„ 9	95·2	100
American 29 ..	131·3	10	„ 12	117·8	100
Belgian 30 ..	19·8	5	„ 26	6·4	10
			American 27 ..	14·8	10
			„ 28 ..	29·6	20
			French 31	18·3	10

It therefore became of fundamental importance to discover whether tuberculins found to be equal (within the error of the test) to standard by the official Frankfort test on guinea-pigs could vary widely in their content of the active principles of "tuberculin," the substance used for the detection of tuberculosis in man and animals. A joint investigation was therefore arranged. It was agreed that one of the workers at the Wellcome Physiological Research Laboratories should choose from materials available at the laboratories three tuberculins, and should hand them to the various workers, each of whom would know nothing of the value of the tuberculins or of results obtained by the other workers until he had made his final report.

The three tuberculins were 1366, a Koch's old tuberculin made on the ordinary glycerine veal broth medium, 1360, and 1232. These two latter had been made in the ordinary way from strains of *B. tuberculosis* grown as a film on glycerine veal broth in the expectation that good tuberculin would result. When the filtrates had been concentrated and batches of the desired Koch's old tuberculin had resulted, the "tuberculin" failed to pass the ordinary animal test. Further examination by the various guinea-pig tests indicated that the "tuberculin" values of these two batches were very low—approximately 2 to 5 per cent. and 5 to 10 per cent.

In order that the various workers should use the same technique, Dreyer and Vollum arranged a full demonstration of the details of the precipitin test at Oxford. This enabled Okell, with the help of Miss G. I. Steed, to carry out three precipitin tests on the three tuberculins. These latter workers with their limited experience of this test would not attach undue importance to the accuracy of their figures obtained by the precipitin method. However, in their three tests they always placed the three tuberculins in the same order of merit (Col. 7) as Dreyer and Vollum (Col. 6, Table II.).

Cummins carried out the testing of the three tuberculins on a number of patients. He decided to test out these tuberculins in a series of dilutions as formerly suggested by Dreyer and according to the technique described by himself (1924). This same technique was used by Dreyer and Vollum in the "human cutaneous test" described in their paper

Table II.

Col. 1.	Col. 2.	Col. 3.	Col. 4.	Col. 5.	Col. 6.	Col. 7.
Tuberculins.	Original values (Okell and Parish).	Parish.	Cummins.	Buxton.	Dreyer and Vollum.	Okell and Miss Steed.
	T.B. guinea-pigs, subcutaneous. von Pirquet and intracutaneous.		Human patients: cutaneous test.	Tuberculous cows: intradermic test.	Floeculation test.	
1232	5–10	About 5.	Both weak and ineffieient.	Poor.	100	100
1360	2½–5	About 2½.		Useless.	59	50
1366	100 = Frankfort standard.	100	Thoroughly reliable; powerful tubereulin.	Full potency.	14	12

Col. 1.—The original batch numbers of the tuberculins.

Col. 2.—These values were, prior to the present joint investigation, given by Okell and Parish as the results of many tests by the subcutaneous, intradermic, and von Pirquet methods applied on many oceasions over a period of about 18 months. The values obtained by the three methods on the tubereulous guinea-pig agree fairly closely.

Col. 7.—Preeipitin tests by Okell and Miss Steed. Vollum provided a generous supply of the preeipitating serum. Okell and Miss Steed report "on preeipitin test 1232 markedly better than 1360 or 1366. 1360 is definitely better than 1366." The arbitrary values given are the averages of two series of readings amongst whieh rather wide fluctuations oceurred. These figures eorrespond fairly elosely to those in Col. 6.

Twenty patients affected with various forms of tuberculosis, and suitable controls, were used in the investigation. An examination of the records of the local reactions led Cummins to report before he had any further details relating to the tuberculins, that "1366 is a very efficient preparation, superior in every way to 1360 and 1232. The two latter samples are indeed so poor in reaction value as to be useless and misleading for clinical purposes."

Buxton tested the three tuberculins on tuberculous cows in various dilutions by the "repeat intradermic test" (as described by himself (1925)). His conclusions are given in Col. 5 of Table II.

The results of the joint investigation are given in Table II. It will be seen that the official Frankfort or "guinea-pig" method, the intracutaneous and von Pirquet tests in guinea-pigs, the cutaneous method in human beings, and the intracutaneous test on tuberculous cows all agreed in indicating that 1366 was a satisfactory tuberculin, and that 1360 and 1232 were practically worthless, while the precipitin method suggested that if 1232 was a tuberculin of precipitin value 100, 1366 had a value of only 12 and 1360 of 50.

With reference to the precipitin method. the results of Mueller (1925) and of Laidlaw (1925) indicate that the active principle of "tuberculin" and the substances precipitable by an antiserum, are probably two distinct substances, separable by chemical methods.

Summary.

1. The precipitin test does not necessarily measure the active principle of tuberculin on which its use in the various methods of diagnosis of tuberculosis depends.

2. Satisfactory agreement in precipitin unit value was obtained by two different groups of workers by the precipitin test. This test apparently measures with a considerable degree of accuracy a substance probably other than the active principle of tuberculin, and presumably the interesting precipitable substance of Mueller and Laidlaw.

3. The "tuberculin" value of tuberculins which have been standardised by the official Frankfort method cannot in all cases be deduced from the precipitin test.

4. The results obtained by subcutaneous, intracutaneous, and von Pirquet tests on guinea-pigs and by the tests on tuberculous human beings and cows were in agreement. By these three methods a tuberculin of standard strength and two others of values approximately 10 and 5 per cent. of standard were placed correctly in order of merit.

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